With the Authors' Compliments.

CAB INTERNATIONAL
MYCOLOGICAL INSTITUTE
LIBRARY
19 FEB 1992
GREGORY, F.G.
1928

[*From the* PROCEEDINGS OF THE ROYAL SOCIETY, B, Vol. 102, 1928.]

A Quantitative Study of the Course of Fungal Invasion of the Apple Fruit, and its Bearing on the Nature of Disease Resistance.

Part I. *A Statistical Method of Studying Fungal Invasion.*

Part II. *The Application of the Statistical Method to certain Specific Problems.*

Part I By F. G. GREGORY, D.Sc., AND A. S. HORNE, D.Sc.
Part II By A. S. Horne D.Sc., and F. G. Gregory, D.Sc.

COMMONWEALTH MYCOLOGICAL INSTITUTE
3 MAR 1955

[*Reprinted from the* PROCEEDINGS OF THE ROYAL SOCIETY, B, Vol. 102.]

A Quantitative Study of the Course of Fungal Invasion of the Apple Fruit and its Bearing on the Nature of Disease Resistance.—Part I. *A Statistical Method of Studying Fungal Invasion.*

By F. G. GREGORY, D.Sc., and A. S. HORNE, D.Sc.

(Communicated by Prof. V. H. Blackman, F.R.S.—Received March 31, 1927.)

(From the Department of Scientific and Industrial Research, and the Department of Plant Physiology and Pathology, Imperial College of Science and Technology, London.)

CONTENTS.

	Page
I. Introduction	427
II. The Material used	428
III. The Experimental Method	429
IV. A Method of Treating the Primary Data for Statistical Purposes	429
V. The Distribution of Radial Advance and Percentage Weight of Rotted Tissue in Random Samples of Apples	434
VI. Evidence for the Specific Nature of Resistance to Fungal Invasion	439
VII. Summary	442

I.—*Introduction.*

In the course of investigations on the incidence of disease in apples stored at low temperatures, which have been in progress since 1920, it soon became evident that a regular series of fungal species appeared successively during the period of storage. It seemed desirable to investigate the problem more closely with a view to disentangling the factors concerned in the succession. The first method employed for the purpose consisted in taking a periodical census of the population of apples and noting the number of apples "invaded" and "not invaded." The need for improvement on this method of studying fungal invasion became clear in the course of the investigation, and led to the development of the technique, described in the present paper. The method of the periodical census has been utilised by M. N. Kidd (1) who has constructed curves of the progress of fungal invasion and from them has derived "mortality curves." The interpretation of these "mortality curves" is, however, somewhat difficult, representing, as they do, not only the effect of varying resistance in the apple, but also the interaction of this factor with the chances of casual infection, which includes the varying powers of penetration possessed by different species of fungi. The distribution of the two classes of apples "invaded" and "not invaded," depends on the interaction of three factors:

(1) the conditions of infection, (2) the progress of invasion after infection, and (3) the final stage of invasion. The bearing of these factors on the problem of invasion of apples will now be briefly considered.

(1) Unless precautions are taken to ensure equal chances of infection by utilising a sound technique of inoculation the problem of the incidence of disease in stored apples is confused by factors such as the unequal distribution of infecting organisms in individual apples of the population, the unequal distribution of spores on individual apples in relation to the distribution of the lenticels or other points of entry, and so on. The method employed in the course of this investigation has already been described (2) and has proved itself adequate.

(2) It is the problem of the progress of disease after infection which will be mainly dealt with, and the method of study will be fully described subsequently in this paper. It is clear, however, that the method previously employed takes no account of the degree of infection, and indeed the assessment of the virulence of two species or strains of fungi will depend on the time at which the census is taken, and should the onset of the invasion be much delayed after the entry of the fungus the main progress of the invasion may be altogether missed.

(3) In the course of the investigation it became clear that after the primary infection the further development of the fungus may be arrested for a long period of time although the fungus remains viable. Such arrest of invasion, which has been called "interference" in this paper, was previously noticed in studying the "spotting" of apples (3) and has been met with on several occasions in the course of this work. This "interference" must be taken into account in assessing the virulence of particular species or strains. The phenomenon is always confined to a varying proportion of the total number of apples in which rotting is checked in the early stages of invasion, while in the remainder invasion proceeds normally to completion. Unless the course of the invasion is followed in such cases an entirely erroneous conclusion will be reached, since by mere inspection apples showing "interference" would be placed in the class "not invaded."

II.—*The Material Used.*

The varieties of apples used throughout this investigation were Cox's Orange Pippin from Burwell, Cambridgeshire and Bramley's Seedling from six localities.

The fungi principally used were originally isolated from apples as previously described (3, 4) and consisted of *Botrytis* sp., *Cytosporina ludibunda*, *Fusarium* sp. (5), *Pleospora pomorum* and *Polyopeus aureus*.

III.—*The Experimental Method.*

At the time of gathering, apples were selected as far as possible of uniform size and free from obvious blemishes. They were then infected at two opposite equatorial points by the standard method already described (2) and transferred as soon as possible to the Low Temperature Station, Cambridge, where they were kept under constant conditions of temperature and humidity. For this work two temperatures were mainly employed, viz., 3° C. and 12° C. From time to time batches of inoculated apples were despatched from Cambridge to London for examination. The apples were then individually weighed and the superficial diseased areas on the two sides were either measured, or the diseased tissue removed and weighed. The measurements taken of the two diseased areas were the mean diameters, obtained by averaging the lengths of two diametral arcs at right angles. The weight of the diseased tissue was obtained by carefully scooping out the decayed areas on each side in turn and weighing the apple after each operation. When invasion was nearly complete it was not possible to record the weights in the two sides separately.

In cases where "interference" had occurred it was found that the rotten tissue had dried out and hollow spaces were left inside the apple. In order to estimate the extent of the invasion prior to drying in such cases, the volume of the hollow was measured by filling with water from a burette and so obtaining the volume. The specific gravity of the apple tissue was then determined and these volumes were converted into weights.

In all doubtful cases where contamination was suspected the causal organism was re-isolated, and unless the original infecting fungus was recovered unassociated with others, the apple was rejected.

The results here recorded are based on a selection from experimental data involving 15,000 separate inoculations, which were carried through by competent helpers.* The main body of results will be dealt with in a second paper (Part II).

IV.—*A Method of Treating the Primary Data for Statistical Purposes.*

The kinds of problems which will be dealt with in the second part of this paper are as follows :—

(1) Comparison of the infecting power of different species and strains of fungi in apples of a given variety.

* The authors' thanks are due to Mrs. Cartwright, Mrs. Harvey and Mrs. Horne for the skilful and efficient way in which this work was done.

(2) The resistance to invasion of different varieties of apples to certain fungal species and strains.

(3) The change in resistance undergone by apples during storage and during the course of invasion by fungi.

The infecting power of the fungi must be assessed from the amount of damage done in a given time, or the time taken to do equivalent amounts of damage. Three possible measures of the damage done will be considered : (*a*) the superficial area of discoloration ; (*b*) the weight of rotten tissue produced in a given time, either as absolute weight or as percentage of the weight of the apple ; (*c*) a measure derived from the weight of rotten tissue, which will be called "radial advance."

The method of assessing the amount of damage by the measurement of superficial areas was used for one experiment only, before the weighing method had been tried. It was abandoned, since it was found that the area of discoloration apparent on the surface was in many cases smaller than the real affected area, a thin superficial layer of sound tissue being left just below the skin.

The weight of rotten tissue may be accurately determined, but the following considerations indicate, however, that the weight of rotten tissue is not an ideal measure to employ.

It has been assumed that infection proceeded from two points at the opposite ends of a diameter in the equatorial plane of the apple, and that the advance has followed spherical shells centred at the points of infection. The further assumption has been made that the apple is spherical in form and is uniform throughout. If the apple were a sphere of unlimited size and invasion proceeded at a uniform rate then the mass of decayed tissue would increase as the cube of time.

Actually the apple approximates to a sphere of very limited size compared with the infected volume and hence only in the very early stages would this relation hold. The curve of total mass of decayed tissue plotted against time is in fact of complex sigmoid form. It can be shown by geometry that in a sphere of radius R, when the front of the invaded zone has reached a certain distance from the point of inoculation, where x is the distance divided by the length of the radius of the sphere, the volume (V) of affected tissue may be expressed by the equation

$$V = \frac{\pi}{12} R^3 x^3 [8 - 3x],$$

when $x = 2$ this becomes $V = \frac{4}{3}\pi R^3$ and is the total volume of the sphere. The distance from the point of inoculation to the front of the invasion, expressed as

a fraction of the radius, will subsequently be called "radial advance." Taking the total volume of the sphere as 1, the proportion (v) of the total volume affected will be given by

$$v = \frac{x^3(8 - 3x)}{16}.$$

This relation holds only when invasion proceeds from one point on the surface of the apple. When two points of infection are considered, the proportion of the total volume affected will be double the amount calculated from the formula in the early stages, until the advancing fronts of invasion meet at the centre of the sphere, when 63 per cent. of the total volume has been invaded. After

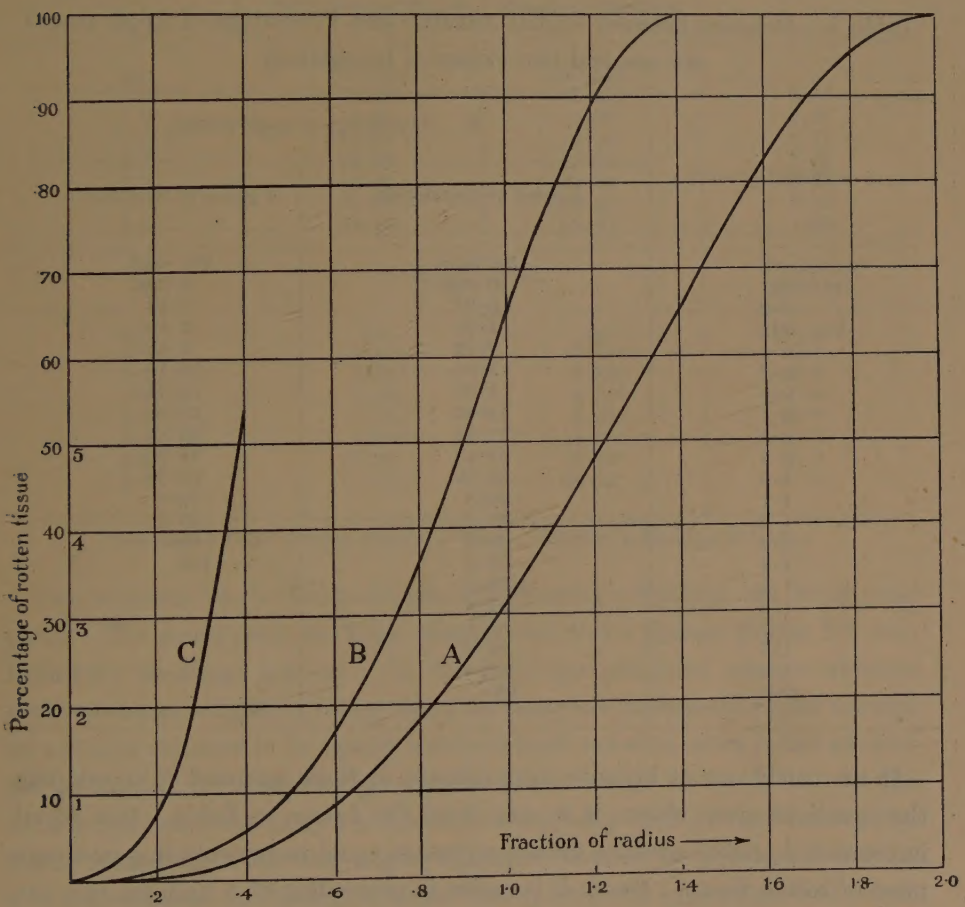

FIG. 1.—Curve showing the relation between the percentage of rotted tissue and radial advance, expressed as a fraction of the radius. A, for apples inoculated at one point. B, for apples inoculated at two points. C, early part of curve B enlarged 10 times.

this, overlapping will occur and a correction has to be made for the volume of overlap, as proportion of total volume of sphere, given by the expression

$$v' = \frac{(x-1)(4x^2-2x-2)}{4}.$$

Fig. 1 shows the curve of percentage volume of mass rotted (v) plotted against x (x = radial advance) for a sphere inoculated at one and two points respectively, and in Table I the values are given of the percentage mass (V) rotted for increasing radial advances. It is evident that complete rot corresponds to a penetration of 1·4 ($=\sqrt{2}$) times the radius.

Table I.—Relation between Radial Advance and Percentage of Apple rotted for one and two Points of Inoculation.

x = Radial advance.	V = Percentage of apple rotted.	
	1 point of inoculation.	2 points of inoculation.
	Per cent.	Per cent.
0·1	0·048	0·096
0·2	0·37	0·74
0·3	1·20	2·40
0·4	2·72	5·44
0·5	5·08	10·16
0·6	8·37	16·74
0·7	12·65	25·30
0·8	17·92	35·84
0·9	24·15	48·30
1·0	31·50	63·00
1·1	39·1	76·6
1·2	47·5	87·2
1·3	56·3	96·4
1·4	65·2	100
1·5	73·8	
1·6	82·4	
1·7	89·1	
1·8	94·8	
1·9	98·6	
2·0	100	

If the conditions of invasion approximate to those assumed in developing the equations given above, it is clear from the figures in Table I that equal increments in radial advance do not represent equal increments in percentage mass of rotten tissue. Hence if invasion is proceeding at a uniform rate the relation of percentage amount of rot, hence also the weight of rotten tissue, produced in two equal increments of time will depend on the particular stage of invasion at which the comparisons are made. Further the relations of amounts

of rotten tissue will depend on the size of the apples inoculated, and if relative virulence is estimated by comparison of weights of tissue rotted, errors will be introduced unless a correction for size of apples is made. An example of the magnitude of the errors liable to be introduced by the effect of size of apple is shown in Table II.

Table II.—Errors introduced into Comparison of Invasion by Effect of Size of Apple.

Radial advance in cm.	Percentage weight of rot.		Ratio.
	Cox's Orange Pippin.	Bramley's Seedling.	
cm.	Per cent.	Per cent.	
0·5	0·656	0·254	2·58
1·0	4·88	1·92	2·54
1·5	15·05	6·10	2·47
2·0	32·38	13·58	2·38
2·5	56·84	24·78	2·29
3·0	87·14	39·80	2·19
3·5	98·62	58·40	1·69
	Weight of rot.		Percentage difference.
cm.	cm.	cm.	Per cent.
0·5	0·115	0·119	3·4
1·0	0·858	0·896	4·4
1·5	2·645	2·846	7·6
2·0	5·691	6·336	11·3
2·5	9·990	11·561	15·7
3·0	15·32	18·57	21·2
3·5	17·33	27·25	57·2

Mean radius Cox's Orange Pippin, 2·6 cm.; Bramley's Seedling, 3·6 cm.

Two varieties, Cox's Orange Pippin and Bramley's Seedling, are being compared. The mean radii for these varieties are Cox's Orange Pippin 2·6 cm., Bramley's Seedlings 3·6 cm. In the table are presented relative absolute and percentage weights of rotten tissue for these two varieties of apples invaded by a fungus assumed to be equally active in both varieties, when radial advance has proceeded for distances given in the first column of the table. In this hypothetical case the fungus is known to be equally virulent in either variety, yet by comparing either the weights of rotten tissue or the percentage weights a wrong estimate of virulence would result, and the errors involved would depend on the stage of invasion at which comparison was made.

This effect of absolute size of apples invalidates the use of absolute or percentage weights of rotten tissue in comparing the virulence of a given species

of fungus in apples of the same or different varieties if these differ in absolute size. An ideal measure of invasion should be independent of the size of the apple, and moreover should give equal numerical values for equal intervals of time, if the rate of invasion remains constant. In so far as the assumptions made in deriving the radial advance from the percentage weight of apple affected are justified, radial advance will successfully eliminate the effect of absolute size, and will give equal numerical values for equal intervals of time if the rate of invasion of the fungus is constant. The primary data derived from weight of rotten tissue can therefore by conversion to radial advance be used to compare the course of invasion by fungi even where the rate of invasion differs in two cases, or apples of different sizes are used. Further, any change in resistance will be reflected in a rise or fall in magnitude of the rate of radial advance, and in no other way could changes in resistance during invasion be followed.

The method of dealing with samples which was finally adopted was as follows. The sum of the weights of rotted tissue recorded from the two sides was first converted into percentage of the total weight of the apple. From the curve in fig. 1 the percentage data were converted into percentages of radial advance. From the weights of the individual apples and the specific gravity characteristic for the variety of apple, the mean radius in centimetres of the apples constituting the sample was calculated, and this figure multiplied by the mean radial advance measured the penetration in centimetres. In this way apples of different sizes could be compared and the rate of invasion calculated over different periods of time and with different fungal species. The probable error of the figures obtained from each operation was calculated, and hence the probable errors of the final figures for the rate of invasion were known.

V.—*The Distribution of Radial Advance and Percentage Weight of Rotted Tissue in Random Samples of Apples.*

In practice the progress of invasion in a uniform set of apples by a given species of fungus is followed by taking samples at different times and determining the amount of rotten tissue in each apple by the method of weighing already indicated. Although the apples are originally selected as uniform as possible, yet in any one sample large variations in weight of rotten tissue from the individual apples are invariably found. These differences in the amount affected may be attributed to variations in resistance to invasion in individual apples. Considering two apples of different resistance inoculated with the same fungus, it may be assumed that the radial advance will be inversely proportional to the

resistances of the two apples.* From the distribution of radial advance in a set of apples some information may be derived as to variation in resistance in individual apples in a sample. Since the resistance cannot be directly measured, its nature being unknown, and its magnitude being assumed to be inversely proportional to rate of radial advance, any conclusions as to resistance will depend for their value on the validity of the assumptions made in deriving radial advance from the experimental data of weights of rotten tissue. It may be well therefore to present evidence that the distributions of weights of rotten tissue in samples of apples of varying resistance actually conform with those expected if the conditions of invasion approximate to those postulated in the geometrical treatment of the invasion of a uniform sphere.

In fig. 2 is presented a set of distribution curves embodying the data from five

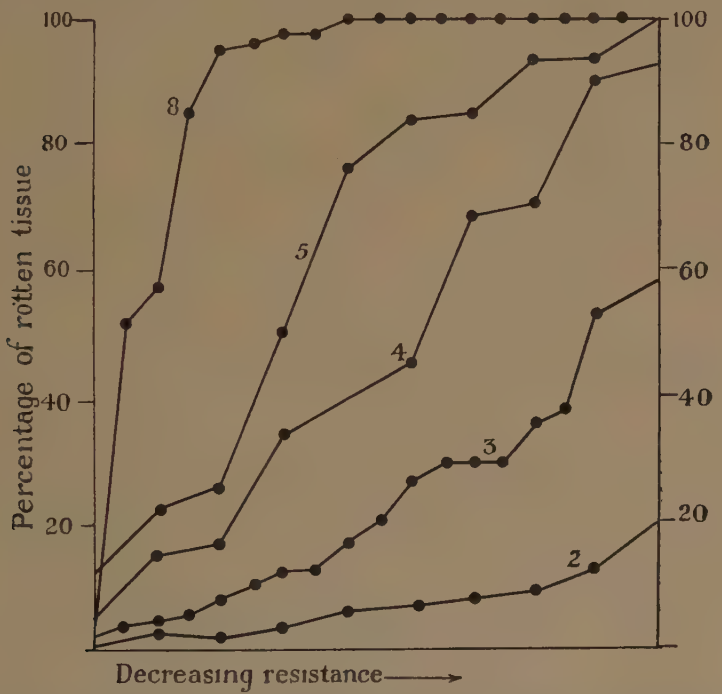

FIG. 2.—Distribution curves of percentages of weight of tissue rotted in samples of apples arranged in serial order of increasing weight, for comparison with the theoretical distributions in fig. 3.

2 = Cox's Orange Pippin 12° C., 1924–25. *Fusarium* strain B11.
3 = Bramley's Seedling, 12° C., 1924–25. *Fusarium* strain C21.
4 = Cox's Orange Pippin, 12° C., 1924–25. *Fusarium* strain A.
5 = Cox's Orange Pippin, 12° C., 1924–25. *Fusarium* strain C1.
8 = Cox's Orange Pippin, 12° C., 1925–26. *Botrytis* sp.

* This will only be true up to the limit at which the fungus is growing at the maximum rate conditioned by internal factors.

selected experiments. The ordinates are the percentage weights of rot from each individual apple in the sample, which have been arranged in serial order along the abscissa. The various curves indicate the distribution of percentage

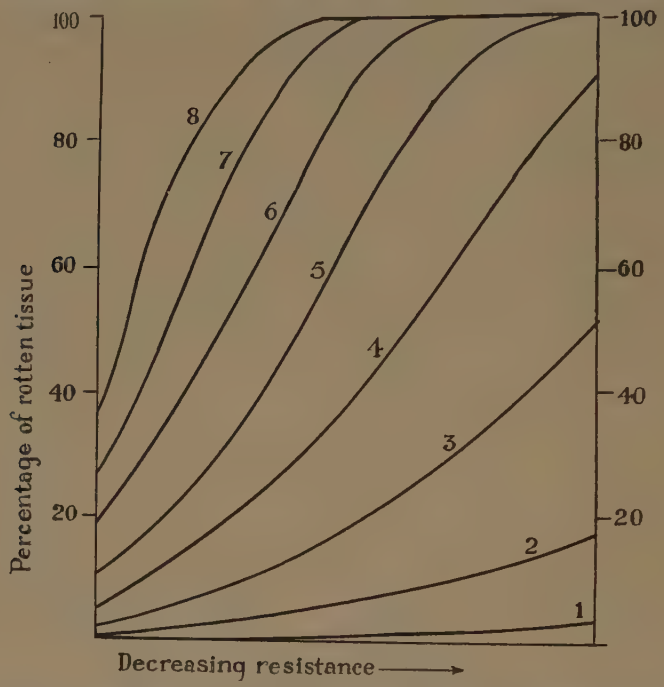

Fig. 3.—Theoretical distribution curves of percentage weight of tissue rotted, arranged in serial order, for apples of varying resistance after successive equal intervals of time (1–8).

weight of rotten tissue in individual apples for samples in which the invasion has proceeded to varying extents towards completion. These curves may be compared with those in fig. 3 which are purely theoretical and were constructed in the following manner :—

Five hypothetical apples were considered characterised by rates of radial advance, for an assumed fungus, 0·3, 0·25, 0·20, 0·15 and 0·10 of the radius per unit of time respectively. The reciprocals of these values would give the relative magnitudes of the resistances of these hypothetical apples. The apples have been arranged in serial order of increasing radial advance, the apple of highest resistance being on the left of the diagram, the others being placed in order of decreasing resistance. Taking times 1, 2, 3, 4, etc., the radial advances after successive periods of time were found, and from the curve in fig. 1 these values of radial advance were converted into the corresponding percentages of rotten tissue.

It is the values of percentage of apple rotted which are presented as curves in fig. 3, each curve being marked with the period of time elapsed. The curves thus represent distribution curves of weight of rotted tissue, arranged in serial order for a given set of apples, after the lapse of varying periods of time; or they may represent equally well distribution curves which would be obtained after a given period of time from sets of apples, all of the same kind, but infected with fungi of different virulence. The resemblance of the theoretical and experimental distribution curves* lends some probability to the validity of the simplifying assumptions introduced into the geometrical considerations of the problem of invasion. Frequency distributions of percentage weight of rotten tissue and of radial advance in a large population of apples are shown in figs. 4 and 5.

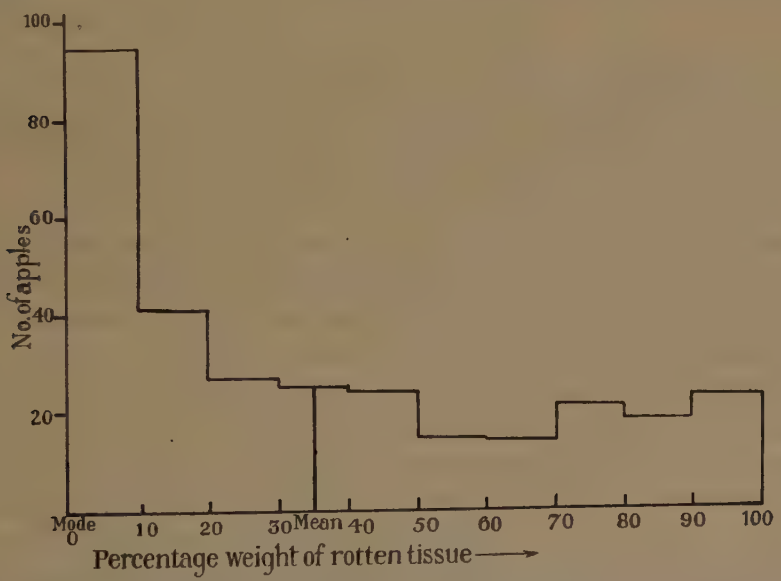

FIG. 4.—Frequency distribution of invasion in a set of 301 inoculated apples, measured in terms of percentage weight of rotted tissue.

Cox's Orange Pippin { 3° C., 1925–26 / 12° C., 1925–26 } *Botrytis.*

" " { 3° C., 1925–26 / 12° C., 1926–27 } *Cytosporina.*

Bramley's Seedling { 12° C., 1926–27 / 20° C., 1926–27 / 20° C., 1927–28 } *Cytosporina.*

* A rigid test of the similarity of the curves in figs. 2 and 3 would require an independent measure of the resistance in individual apples. The only assumption which has been made is that resistance does vary in individual apples, and that the individuals in a sample can be arranged in a serial order of increasing resistance.

The data were derived from 301 apples in five samples, three sets of Cox's Orange Pippins (177 apples), and two sets of Bramley's Seedlings (124 apples).

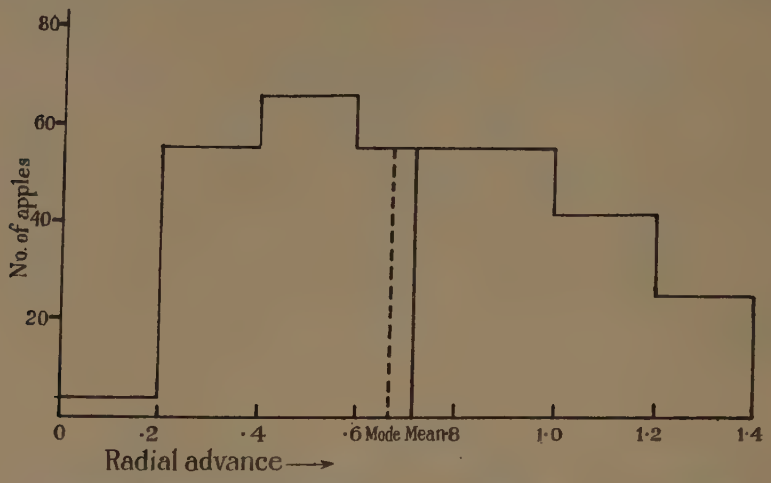

Fig. 5.—Frequency distribution of invasion in the same set of 301 inoculated apples measured in terms of radial advance.

The figures for percentage weight of rotten tissue were divided into 10 classes each covering a range of 10 per cent., and a frequency distribution was made of the number of apples falling into each class. This is presented in fig. 4. The percentage weights were then converted into radial advance, and the figures thus obtained divided into seven classes, each covering a range of 0·2 radial advance. The numbers of apples in each class are shown in fig. 5.

The distribution of percentage weights is seen to be highly asymmetrical, while radial advance distribution is only moderately asymmetrical. The standard errors of the means of the two distributions are 5·02 per cent. for percentage weights and 2·23 per cent for radial advance respectively; hence mean radial advance of a sample may be estimated with twice the accuracy of percentage weight of rotten tissue. In addition, therefore, to the advantage which radial advance has over weight of rotten tissue as a measure of invasion in eliminating the effect of size of apple and the stage of invasion reached, the added advantage of greater accuracy is obtained, so that samples of one-half the size may be used without loss of efficiency. Since distributions departing largely from symmetry are statistically inconvenient. the treatment of the results of the experiments to be reported in Part II of this paper would seem to require the choice of radial advance as the working variate.

The distribution of radial advance itself, however, tends to become increasingly

asymmetrical as invasion proceeds towards completion. Increasing numbers of apples will fall into the class 100 per cent. rotten (radial advance 1·4), and the mode of the distribution will shift gradually towards the highest class, in which the majority of apples are finally found, simply because invasion can advance no further. It is evident then that for purposes of comparing the effect of given species of fungi on different varieties of apple, or comparing the virulence of fungal species or strains on the same variety of apple, the sample should be taken not later than the time when one of the series first shows a completely rotten apple.

From the foregoing considerations it is evident that radial advance provides a convenient scale in which to measure the individual resistance of the apple to fungal invasion, and defined in this way resistance is seen to be distributed in a nearly normal manner in a population of apples.

VI.—*Evidence for the Specific Nature of Resistance to Fungal Invasion.*

At this point it may be well to give evidence for the specific nature of variations in resistance in a population of apples. If the resistance within a population of apples varies, it might be expected that difference in resistance within an individual apple would be less than in any two apples taken at random. This has been confirmed by estimating the correlations between radial advance of a given fungus on two sides of individual apples as well as the correlations between weights of rotten tissue derived from the two sides. The data are presented below (Table III). Two varieties of apple have been used at two different temperatures, tested with three fungal species. The correlations for percentage weight of rotten tissue are enclosed in brackets. The correlation coefficients for radial advance are all positive and significant, and are in every case greater than the corresponding values for percentage weight, reflecting the smaller standard deviation of radial advance.

The correlations provide clear evidence of the specific value of resistance in individual apples.

Although the correlations so far presented indicate that the resistance at one pole of an apple tends to be similar to that at the opposite pole, examination of a set of data giving individual weights of the rotten tissue from the two sides reveals striking differences. It seemed possible therefore that the resistance of an individual apple might vary greatly from point to point around the circumference.

To test this point a set of 20 Bramley's Seedling apples was taken and each apple was inoculated at four points equidistant around the circumference. The

results as determined in terms of radial advance after storage for 17 days are given in Table IV.

Table III.—Correlation between Estimates of Invasion on two Sides of Individual Apples.

Season.	Fungus.	Temperature.	Variety of apple.	Correlation coefficient (r).	No. of apples.
		° C.			
1925–26	*Fusarium.* Strain D	12	Bramley's Seedling	(+0·603) +0·874	19
	Botrytis. Sample I	3	Cox's Orange Pippin	(+0·479) +0.598	20
	Botrytis. Sample II	3	Cox's Orange Pippin	(+0·597) +0·655	16
	Cytosporina. Sample II	3	Cox's Orange Pippin	(+0·428) +0·657	16
	Cytosporina. Sample I	3	Cox's Orange Pippin	(+0·610) +0·662	20
1924–25	*Cytosporina*	3	Bramley's Seedling	(+0·556) +0·717	25
			Mean	(+0·546) +0·694	116

Table IV.—Radial Advance at four equidistant Points in Individual Apples Inoculated with same Fungus.

	a.	*b.*	*c.*	*d.*
1	0·26	0·25	0·22	0·28
2	0·28	0·24	0·24	0·27
3	0·41	0·30	0·37	0·38
4	0·37	0·27	0·28	0·23
5	0·41	0·28	0·26	0·27
6	0·43	0·28	0·28	0·29
7	0·39	0·26	0·27	0·30
8	0·37	0·22	0·36	0·24
9	0·44	0·18	0·38	0·33
10	0·47	0·28	0·38	0·28
11	0·54	0·40	0·48	0·29
12	0·38	0·30	0·32	0·28
13	0·46	0·33	0·36	0·32
14	0·50	0·28	0·36	0·28
15	0·46	0·28	0·40	0·36
16	0·60	0·56	0·45	0·40
17	0·54	0·42	0·44	0·37
18	0·41	0·37	0·28	0·33
19	0·52	0·39	0·28	0·39
20	0·62	0·28	0·38	0·42
Mean	0·443	0·309	0·340	0·316

In each case the figures are the radial advances occurring at the four points (*a*, *b*, *c*, *d*), the highest value recorded in each case being placed first (*a*), and the rest in serial order around the circumference. The mean values show an alternation of high and low values. The mean value of the figures in column *c* is necessarily lower than the mean value of those in column *a*, since the highest values have arbitrarily been selected and placed first. From these figures it is possible to test more rigidly the relation of the resistances of opposite sides of individual apples.

In order to test this point correlations were calculated between radial advance in column *a* and the values of each of the other columns in turn. The results are as follows :—

$$\left.\begin{aligned} r_{ab} &= +0{\cdot}680 \\ r_{ac} &= +0{\cdot}742 \\ r_{ad} &= +0{\cdot}679 \end{aligned}\right\}.$$

All these correlations are positive and highly significant, indicating again that the resistance within an apple tends to a specific value. To test whether the alternation in high and low values in columns *b*, *c* and *d*, as indicated by the means, is significant, the effect of the variation in resistance at the point (*a*) was eliminated, so that the relations of the resistances at the points (*b*), (*c*) and (*d*) were found for apples of equal resistance at the point (*a*). The partial correlations $r_{bc.a}$ and $r_{cd.a}$ were therefore calculated with the following results :—

$$\left.\begin{aligned} r_{bc.a} &= -0{\cdot}102 \\ r_{cd.a} &= -0{\cdot}159 \end{aligned}\right\}.$$

These values are quite insignificant and indicate that in apples of equal resistance at one point no real difference on the average exists between the resistance at any two other points round the circumference. This result was confirmed by taking differences between the figures in columns *b* and *c* and *c* and *d*, and testing by Student's method. The evidence for the specific value of resistance in individual apples is thus seen to be cumulative.

The final test made was to inoculate apples at two opposite points (*a* and *c*). The invasion was allowed to reach a certain stage and then the rotten tissue was removed and weighed, and the holes sealed with wax. The apples were then reinoculated at the alternate points (*b* and *d*) and after a time the amount of rot determined as before. The results are given below in Table V.

Table V.—Radial Advance following two Successive Inoculations of same Individual Apples.

	Radial advance after first inoculation. 16 days.	Radial advance after second inoculation. 10 days.
1	0·47	0·46
2	0·46	0·31
3	0·44	0·30
4	0·40	0·30
5	0·34	0·26
6	0·34	0·29
7	0·34	0·28
8	0·27	0·22
9	0·24	0·21
10	0·24	0·22

A correlation coefficient was determined between the radial advance resulting from the first and second inoculations, respectively. The value found was $r = +0{\cdot}816$, which agrees very well with the values previously determined for correlation in resistance between the halves of single apples. The value has a significance greater than 100 to 1 (6) and finally proves the specific value of resistance in the individual apple.

There still remains the remote possibility that variations in invasion were due to changes in the fungus. To test this, the organism was re-isolated from apples showing extreme variations in attack (100 per cent. and 0·4 per cent. of rot). No marked differences could be detected in the vigour of the fungus as measured by its growth rate on a standard synthetic medium; and such differences as were found bore *no relation to the amount of rot at the point from which the fungus was re-isolated.*

The results obtained from further applications of the statistical method described in this communication will be presented in the second paper of this series, when certain specific problems connected with the resistance of apples to fungal invasion and the virulence of fungal strains will be considered.

VII.—*Summary.*

1. A standard technique is described for studying the invasion of apples of different varieties inoculated with various species of fungi. The invasion has been studied during various periods of storage life and at different temperatures.

2. The primary data are derived from upwards of 15,000 inoculations and consist of the weights of rotted tissue derived from individual apples. It is shown that, since invasion occurs in a sphere of limited size, a complex relation

holds between the weight of tissue rotted and the penetration of the fungus. In consequence of this the weight of rotted material obtained during two consecutive equal periods of time will not be equal, even though the penetration of the fungus is at a uniform rate. A method has been devised which takes into account this complex relationship, and a new measure "radial advance" is calculated which is constant over equal intervals of time when invasion is proceeding at a uniform rate, irrespective of the stage of invasion reached.

3. The percentage weights of rotted tissue derived from individuals in a sample of apparently uniform apples are found to vary over a wide range. The distribution of percentage weights with a given fungus is shown to be highly asymmetric, while the same data converted into radial advance give a distribution only moderately asymmetric. The standard error of the mean of radial advance is shown to be less than half that of percentage weights, indicating a great gain in accuracy in the use of radial advance as a measure of invasion.

4. The rate of radial advance provides a convenient scale in which to measure the resistance to invasion in individual apples.

5. It is found that each individual apple in a sample has a characteristic resistance. This is shown by :—

(1) The high positive correlation obtaining between the radial advance at the opposite sides of individual apples inoculated at two or four points :—

$$r = + 0{\cdot}694 \text{ (2 points, 116 apples).}$$
$$r = + 0{\cdot}742 \text{ (4 points, 20 apples).}$$

(2) By the high correlation found to hold between the radial advances obtained from two successive inoculations of the same individuals by the same fungus :—

$$r = + 0{\cdot}816 \text{ (10 apples).}$$

LITERATURE CITED.

(1) Kidd, M. N. "The Mortality Curve in a Population of Apples," 'Rep. Food Investigation Board, 1924.' Department of Scientific and Industrial Research, London.

(2) Granger, K., and Horne, A. S. "A Method of Inoculating the Apple," 'Annals of Botany,' vol. 38, p. 213 (1924).

(3) Horne, A. S. "Diagnosis of Fungi from 'Spotted' Apples," 'Journ. of Botany, vol. 58, p. 238 (1920).

(4) Horne, A. S., and Horne, E. V. "Mycological Studies, I. On the 'Spotting' of Apples in Great Britain," 'Annals of Applied Biology,' vol. 12, p. 183 (1920).

(5) Brown, W., and Horne, A. S. "Studies of the Genus Fusarium, I. General Account," 'Annals of Botany,' vol. 38, p. 379 (1924).

(6) Fisher, R. A. "Statistical Methods for Research Workers," Edinburgh, Table V, p. 174 (1925).

A Quantitative Study of the Course of Fungal Invasion of the Apple Fruit, and its Bearing on the Nature of Disease Resistance.—Part II. *The Application of the Statistical Method to certain Specific Problems.*

By A. S. Horne, D.Sc., and F. G. Gregory, D.Sc.

(Communicated by Prof. V. H. Blackman, F.R.S.—Received March 31, 1927.)

(From the Department of Scientific and Industrial Research and the Department of Plant Physiology and Pathology, Imperial College of Science and Technology, London.)

CONTENTS.

	Page
I. Introduction	444
II. The time sequence of invasion for selected fungal species	445
III. Comparison of the virulence of certain *Fusarium* strains	451
IV. Comparison of variation in resistance shown by different populations of Bramley's Seedling apples	459
V. Concerning resistance to fungal invasion in apples	461
VI. Summary	465

I.—*Introduction.*

In the previous paper (1) a method was developed for studying quantitatively the process of invasion of apples by fungi inoculated into them. It was there shown that the method is capable of yielding a measure of the amount of invasion (radial advance) which is independent of the size of the apple used and of the time of duration of the experiment. The distribution of radial advance in populations of apples was studied and it was shown (1) that the distribution of radial advance* (in terms of which resistance to invasion is measured) is not highly asymmetrical if measured at the right stage of invasion, (2) that an individual apple is more uniform with respect to resistance than are any two apples selected at random. It is shown in the course of the present paper that by utilising radial advance as a measure of invasion the change in resistance to the attack of fungal species during the period of storage can be followed. A comparison is made of the virulence of certain fungal species or strains and of the variation in resistance to fungal invasion shown by different populations of given varieties of apples during storage. The significance of these comparisons has been estimated by the calculation of probable errors, which was shown to be

* Resistance has been assumed to be inversely proportional to the rate of radial advance.

legitimate in the previous paper. Finally the bearing of the results obtained on the problem of disease resistance is discussed.

II.—*The Time Sequence of Invasion for Selected Fungal Species.*

Some information had already been obtained in 1921–22 on the behaviour of certain selected fungi. The results attained at that time were based on periodic counts of the number of apples showing externally marked signs of invasion (using samples of 10 apples). In fig. 1 which is a selection of curves

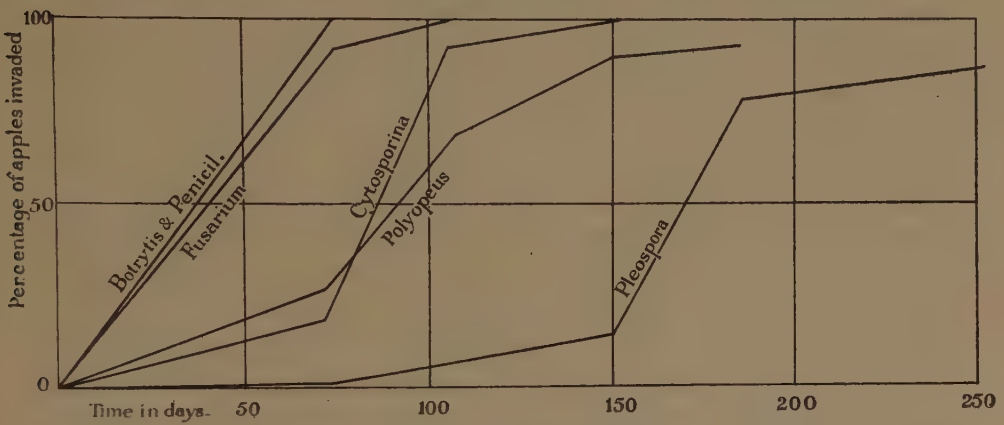

FIG. 1.—Time succession in percentage of apples in a sample showing invasion by various species of fungi. 1° C.

already published (2) the differences in the time of onset of the main invasion are seen to vary greatly for the different species employed, and for this reason some of these species have been selected for further work. As far as the experiment of 1921–22 went it clearly established the fact that the successional invasion of fungal species observed in apples in cold store was not due to a failure of infection, but to a check in development after infection had occurred (3). It seemed desirable, however, to investigate by a more exact method the relation between parasite and host during the period of delay in invasion.

The species selected for further study were (1) *Pleospora pomorum*, (2) *Polyopeus aureus*, (3) *Fusarium* strain A, (4) *Cytosporina ludibunda*, (5) *Botrytis* sp. These were grown on Cox's Orange Pippin apples, obtained from Burwell, Cambridgeshire. One thousand apples were selected and divided into two batches of 500 each. Each batch was inoculated with the five specified fungi (100 apples each). With the first set the inoculations commenced on October 19 and ended on October 23, 1925, using the fungi in the order given above. This set was despatched to the Low Temperature Station, Cambridge, on

October 26 and placed at 12° C. The second set was inoculated exactly one week later, using the same fungi in the same order, and despatched to Cambridge on November 2. These a ples were stored at 3° C.

Each set of 100 apples was divided at random into 5 samples of 20 apples each before despatching the set to Cambridge. Five samples (one for each fungus) were returned to London at more or less regular intervals (fortnightly intervals for those stored at 12° C., monthly intervals for those stored at 3° C.) for systematic examination. The results of the 1925 experiment with Cox's Orange Pippin are given in the following table.

The fungal species are indicated in column 2, the Roman numerals indicating the number of the sample. The time which has elapsed since inoculation is found in column 3. Column 4 gives the mean weights of the apples at the time of sampling, and the calculated mean radius is found in column 5. In column 6 are the mean radial advances derived from the figures for percentage weight of tissue rotted in each apple of the sample, by the use of the curve in fig. 1 in Part I of this paper. The averages of these figures for radial advance are the mean radial advances found in column 6. Column 7 gives the percentage weight of apple rotted, derived from the primary experimental data. The product of the mean radii and the radial advances yield the figures in column 8, and are radial advances in centimetres. Dividing the figures in this column by the number of days elapsed from inoculation the growth rates per day in centimetres, found in column 9, were obtained. The mean growth rates in the intervals between sampling are found in column 10, and were obtained by dividing the increments in radial advance in column 8 by the time elapsed between the two samplings indicated.

The different levels of growth rate exhibited by the various fungi are clearly represented by the figures in columns 9 and 10 of Table I. In the case of *Botrytis*, the growth rate at the higher temperature is approximately double that of the lower, while with *Fusarium* the difference is slightly in favour of the higher temperature. The growth rate of *Polyopeus aureus* at the two temperatures is almost identical. *Pleospora* exhibits a slightly higher growth-rate at the lower temperature and this effect is very marked in the case of *Cytosporina*. With *Fusarium* " interference " occurred at both temperatures.

Averaging the growth-rates at the two temperatures, the order of diminishing values would be *Botrytis*, *Cytosporina*, *Fusarium*, *Polyopeus aureus* and *Pleospora*. This is precisely the order noted in the succession of species already referred to in fig. 1, with the exception of *Fusarium* in which case a weaker strain was utilised for this experiment. The variation in " reaction " exhibited by

Table I.—Rate of Invasion for certain Fungi at different Temperatures.

1. Temperature.	2. Sample.	3. No. of days from inoculation.	4. Mean apple in grams.	5. Mean radius in centimetres.	6. Radial advance.	7. Percentage weight of tissue rotted.	8. Radial advance in centimetres.	9. Growth rate per day in centimetres.	10. Growth rate during interval in centimetres per day.	
° C.										
3	*Botrytis* I	35	63·9	2·63	0·648	24·76	1·704	0·0487	0–I	0·0487
3	,, II	68	65·9	2·65	0·800	36·24	2·12	0·0352	I–II	0·0126
3	,, III	89	54·4	2·49	1·302	91·2	3·24	0·0364	II–III	0·0533
3	,, IV	120	62·5	2·61	1·137	79·8	2·97	0·0248	III–IV	−0·0087
12	*Botrytis* I	19	68·1	2·68	0·850	47·3	2·27	0·119	0–I	0·119
12	,, II	33	67·0	2·67	1·275	89·4	3·40	0·106	I–II	−0·0807
12	,, III	47	67·0	2·67	1·247	86·1	3·33	0·071	II–III	−0·0050
12	,, IV	61	67·0	2·67	1·400	100·0	3·74	0·061	III–IV	0·0293
3	*Cytosporina* I	36	60·69	2·59	0·563	21·0	1·458	0·040	0–I	0·040
3	,, II	70	62·3	2·60	0·816	44·86	2·121	0·030	I–II	0·0195
3	,, III	90	64·3	2·63	0·964	61·0	2·54	0·028	II–III	0·0210
3	,, IV	117	65·7	2·65	1·018	63·8	2·70	0·023	III–IV	0·0059
12	*Cytosporina* I	21	64·8	2·64	0·125	0·9	0·330	0·016	0–I	0·016
12	,, II	35	64·9	2·64	0·164	0·8	0·424	0·012	I–II	0·0067
12	,, III	48	65·5	2·65	0·186	0·9	0·493	0·010	II–III	0·0053
12	,, IV	75	53·9	2·49	0·170	2·0	0·424	0·0057	III–IV	−0·0025
12	,, V	98	57·9	2·56	0·297	11·7	0·760	0·0078	IV–V	0·0146
3	*Fusarium* I	38	68	2·71	0·322	3·1	0·873	0·023	0–I	0·023
3	,, II	71	68·1		0·376	4·6	1·19	0·015	I–II	0·0096
3	,, III	92	70·9		0·38	4·6	1·19	0·013	II–III	0
3	,, IV	118	71·8		0·38	4·6	1·19	0·010	III–IV	0

Table I—(continued.)

1. Temperature.	2. Sample.	3. No. of days from inoculation.	4. Mean apple in grams.	5. Mean radius in centimetres.	6. Radial advance.	7. Percentage weight of tissue rotted.	8. Radial advance in centimetres.	9. Growth rate per day in centimetres.	10. Growth rate during interval in centimetres per day.	
° C.										
12	*Fusarium* I	22	66·89	2·67	0·336	4·4	0·897	0·0408	0–I	0·0408
12	,, II	36	68·63	2·69	0·378	5·5	1·017	0·0282	I–II	0·0086
12	,, III	49	65·4	2·65	0·296	7·0	1·155	0·0236	II–III	0·0029
12	,, IV	88	62·7	2·46	0·520	22·1	1·279	0·0145	III–IV	0·0032
12	,, V	100	47·7	2·39	0·702	36·0	1·678	0·0168	IV–V	0·0333
3	*Polyopeus* I	38	65·8	2·70	0·235	1·4	0·635	0·0168	0–I	0·0168
3	,, II	71	64·8		0·267	2·6	0·720	0·0102	I–II	0·0026
3	,, III	92	67·0		0·282	4·1	0·762	0·0083	II–III	0·0020
3	,, IV	118	77·8		0·501	21·4	1·328	0·0089	III–IV	0·00217
12	*Polyopeus* I	22	77·7	2·76	0·143	0·6	0·394	0·0179	0–I	0·0179
12	,, II	36	70·7		0·235	1·5	0·648	0·0180	I–II	0·0181
12	,, III	48	72·6		0·252	1·4	0·693	0·0144	II–III	0·0038
12	,, IV	77	60·4		0·252	2·3	0·693	0·0090	III–IV	0
12	,, V	99	53·3		0·329	10·7	0·908	0·0092	IV–V	0·0098
3	*Pleospora* I	40	77·6	2·78	0·225	1·1	0·626	0·0156	0–I	0·0156
3	,, II	74	75·9		0·160	0·7	0·446	0·0064	I–II	−0·0053
3	,, III	94	71·9		0·150	0·7	0·417	0·0043	II–III	−0·0015
3	,, IV	120	64·6		0·196	1·1	0·547	0·0046	III–IV	0·0050
12	*Pleospora* I	24	67·2	2·69	0·104	0·4	0·280	0·0117	0–I	0·0117
12	,, II	38	69·3		0·218	1·2	0·586	0·0154	I–II	0·0218
12	,, III	51	63·7		0·185	1·5	0·342	0·0067	II–III	−0·0187
12	,, IV	79	61·9		0·191	2·9	0·514	0·0065	III–IV	0·0061
12	,, V	101	58·3		0·262	3·5	0·705	0·0070	IV–V	0·0086

the *Fusarium* strains is dealt with later. In every case the rate of growth falls in the early part of the experiment, but in the case of *Fusarium* at 12° C. and of *Pleospora* at 12° C. there is an indication of a subsequent rise.

In spite of the part played by sampling errors there is an unmistakable similarity in the drift of growth-rate. The growth-rate falls rapidly in each case until the neighbourhood of the fiftieth day where a minimum occurs, and subsequently in most cases there is an increase in growth-rate. Averaging the values* in all the experiments the following figures are obtained.

Table II.—Mean Values for Growth-rate in time for the Fungi in Table I.

Days from inoculation.	Mean growth-rate in centimetres per day.
20	0·031
30	0·022
40	0·0086
50	0·0072
60	0·0074
80	0·013

The growth-rates have been assumed to be inversely proportional to the resistance of the apple to invasion, and since the growth-rates of all the fungal species employed exhibit the same kind of time-relation, irrespective of temperature, there is possibly a general change in resistance occurring in every case. It is probable that the resistance of the apples was increasing up to the fiftieth day from inoculation, with slight indication of a subsequent fall. If it is assumed that the variation in resistance with time is represented by a curve of the kind shown in fig. 2 and that L 1 and L 2 represent two critical levels of resistance for the two species of fungus F 1 and F 2, then for species F 1 the growth-rate will fall during the interval of time from 0 to T 1 (since during this time the resistance is rising) will reach a minimum somewhere between T 1 and T 2, and will subsequently rise as the resistance once again falls. Such a distribution of growth-rates is seen in the case of *Botrytis* at 3° C. In the case of species F 2 the growth-rate will fall rapidly up to a time T 1 at which point the resistance rises above the critical level. Growth will then cease until such time as the resistance again falls below the critical value. If the portion of the apple which has been already invaded before the first critical point is reached is not negligibly small when growth is checked, subsequent drying out may lead to development of cavities which are characteristic of the phenomenon of "interference." This effect

* The values on the particular days were obtained by interpolation from curves.

was very evident with *Fusarium* at both temperatures and sporadically with *Polyopeus aureus*. In the case of slow-growing fungi the volume of the apple

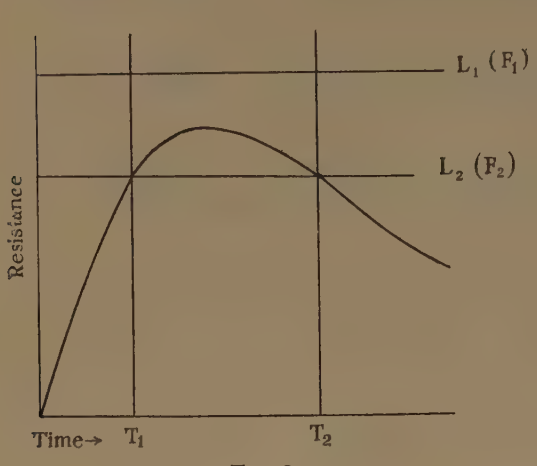

Fig. 2.

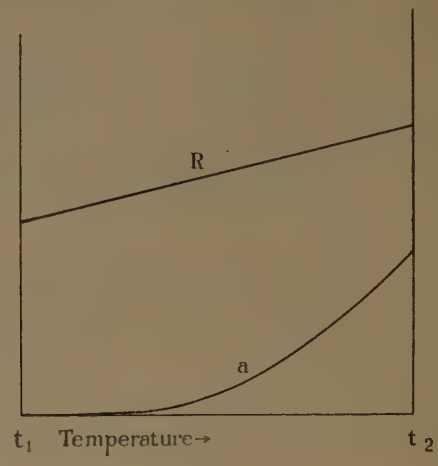

Fig. 3.

affected before the growth ceases would be so small that the cavities would not be evident.

The varying reactions of the fungi at the two temperatures is striking and is difficult to interpret. The relation between parasite and host will depend on the effect of temperature, on the activity of the fungus, and the resistance of the apple. For *Botrytis*, which is a virulent species, the activity is found to increase to double for the temperature rise of 9°, which is near the normal temperature coefficient of growth. In the case of *Fusarium* and the other fungi, the increase in activity is not evident. It is possible that it is masked by the relatively higher resistance of the apple in these cases.

Should the temperature coefficients of resistance and activity be greatly different, the temperature relations may be formally represented by the diagram shown in fig. 3. The rate of invasion has been assumed to be proportional to 1/R, where R is the resistance of the apple. If a is the activity of the fungus we may write

$$V = \frac{a}{R + C}$$

where V is the rate of radial advance and C a constant. It is clear that if the relations indicated in the diagrams hold, $a/R + C$ may have a minimum value between temperatures T 1 and T 2. Such a set of relationships might account for the results obtained with *Cytosporina* at 12° C. and 3° C.; and by suitable

modification of the temperature effect on R, such cases as *Fusarium* and *Polyporus aureus* would be explicable. With our present knowledge it is impossible either to substantiate or refute such a hypothesis, but further lines of work are clearly indicated. The evidence to hand as to the nature of resistance will be discussed later in this paper.

III.—*Comparison of the Virulence of Certain Fusarium Strains.*

An interesting aspect of the problem of invasion of apples is the relative degree of virulence of various strains of a single species of fungus. In the preceding section of this paper different species have been compared, and similar methods will now be applied to various strains of a given species. As some of the strains studied are saltant forms whose origin and previous history are well known, it has been possible to compare these with their parent strains. The practical importance of such comparison in relation to the possible origin of new diseases of apples is evident.

The *Fusarium* strains used for these experiments were originally isolated from diseased apples. The strain A was obtained by Dr. W. Brown of this College, in 1918, from an unknown variety ; the strains B, C, and D from Cox's Orange Pippin, E from Allington Pippin, and F from Sweet Alford, in 1920 by one of the present authors (A.S.H.). The strains B 1, C 1 and C 2 originated from monosporous platings of B and C respectively, and were made in 1921. C 3 was first obtained as a variant from C in tube cultures made from the same monosporous colony of C. The strains B 11, B 111 and D 2 are saltants obtained by Brown, who has recently dealt with the question of saltation in some detail (4). The origin of the strains and saltants is shown diagrammatically below (fig. 4).

The fact that this species of *Fusarium* was capable of parasitising the tissues of the apple was first shown in 1921 (3, p. 369) when, with the exception of *Penicillium* and *Botrytis*, it proved at 1° C. the most active fungus tested in that year (see fig. 1). In 1922 eight strains were tested on the Cox's Orange Pippin and Bramley's Seedling varieties of apples at two temperatures (1° C. and 15° C.). The strains included A, C, C 1, C 2, B 1, D, E, and F. All the strains employed invaded both the varieties at 15° C., but the intensity of attack was much less marked in the case of the Bramley's. At 1° C., the Cox's were slightly invaded by the strains C, C 1, C 2, F, D, B 1, and E. The invasion of the Bramley's at this temperature was negligible (5, p. 39, and 6, p. 59).

As a result of experiments begun in 1923 using the strains C 1, C 2 1, C 3, B 1, B 1 1, A and D on Cox's Orange Pippin at two different temperatures (12° C. and 3° C.) not only was the parasitic activity of these *Fusarium* strains con-

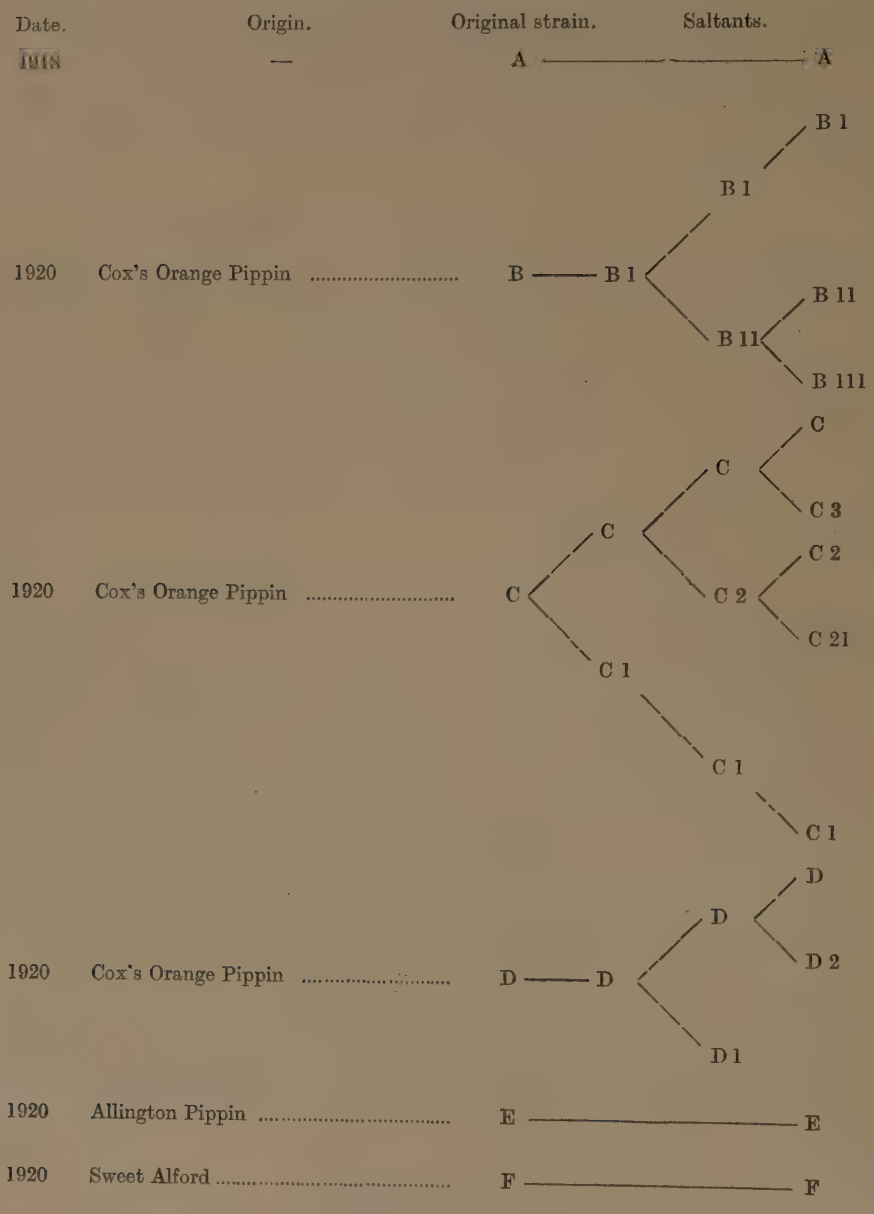

Fig. 4.—Pedigree of *Fusarium* strains.

firmed, but in addition it was clearly evident that they varied in their pathogenicity (6, p. 62). Certain strains such as D 1, C 1, C 21, and B 1 being very active, others (A, C 3) moderately active and others (B 11) very weak. It was found that the strong strains belonged to the group of strains which exhibit

strong mycelial development, whereas the weak strains were those characterised by feeble mycelial development and intense sporulation (see (7)). It was also clear that the saltant B 11 derived from the strongly active strain B 1 differed from the parent in a marked loss of virulence.

In 1924 and 1925 the same experiments were repeated on a larger scale, with Bramley's in addition to the Cox's, using the more exact methods for recording data already outlined in this paper. The results obtained in both years confirm the earlier work, and evidence will be presented to show that the strains can be arranged in serial order with respect to their pathogenicity, and that the sequence is independent of the type of apple used.

The data obtained are summarised in Table III in which the rate of advance in centimetres per day is given in column 6 for each of the strains of *Fusarium* given in column 1. The probable errors for the rates of advance have been calculated and the significance of the differences between strains can thus be tested.

Table III.—Rates of Invasion for certain *Fusarium* Strains in two Varieties of Apple.

1. *Fusarium* strain.	2. Mean weight of apple in grams.	3. Mean radius in centimetres.	4. Radial advance.	5. Radial advance in centimetres.	6. Rate of advance per day in centimetres.
Bramley's Seedling, 1925–26. 12° C. 70 days.					
A	156·2	3·66	0·557 ±0·030	2·04 ±0·15	0·0292 ±0·0021
B 11	153·9	3·65	0·337 ±0·017	1·23 ±0·065	0·0178 ±0·0009
B 111	137·8	3·51	0·356 ±0·021	1·25 ±0·077	0·0179 ±0·0010
C 1	141·0	3·53	0·469 ±0·042	1·66 ±0·15	0·0237 ±0·0021
C 21	145·8	3·58	0·486 ±0·043	1·74 ±0·17	0·0248 ±0·0024
C 3	147·8	3·60	0·523 ±0·046	1·88 ±0·17	0·0296 ±0·0024
D	174·9	3·81	0·595 ±0·036	2·26 ±0·14	0·0322 ±0·0020
D 2	145·3	3·58	0·367 ±0·018	1·31 ±0·068	0·0188 ±0·0010
		Mean P.E. ±0·056			
Bramley's Seedling, 1924–25. 12° C. 103 days.					
A	91·3	3·07	0·378 ±0·041	1·160 ±0·13	0·0113 ±0·0013
B 1	90·9	3·06	0·606 ±0·038	1·854 ±0·13	0·0180 ±0·0013
B 11	115·5	3·05	0·414 ±0·043	1·263 ±0·13	0·0123 ±0·0013
B 111	99·5	3·15	0·131 ±0·027	0·413 ±0·11	0·0040 ±0·0011
C 1	101·2	3·17	0·754 ±0·054	2·39 ±0·17	0·0232 ±0·0017
C 21	106·8	3·23	0·622 ±0·037	2·009 ±0·12	0·0195 ±0·0012
C 3	88·1	3·03	0·652 ±0·048	1·976 ±0·15	0·0192 ±0·0015
D	100·2	3·16	0·714 ±0·046	2·256 ±p·15	0·0219 ±0·0015
		Mean P.E. ±0·048			

Table III—(continued).

1. *Fusarium* strain.	2. Mean weight of apple in grams.	3. Mean radius in centimetres.	4. Radial advance.	5. Radial advance in centimetres.	6. Rate of advance per day in centimetres.
Cox's Orange Pippin, 1924–25. 12° C. 54 days.					
A	66·54	2·66	0·860 ±0·062	2·288 ±0·166	0·0424 ±0·0031
B 1	66·13	2·66	0·952 ±0·080	2·532 ±0·214	0·0469 ±0·0040
B 11	67·59	2·68	0·380 ±0·037	1·018 ±0·099	0·0188 ±0·0018
B 111	64·77	2·64	0·103 ±0·023	0·272 ±0·061	0·0050 ±0·0011
C 1	59·33	2·57	1·025 ±0·063	2·634 ±0·264	0·0487 ±0·0030
C 21	70·06	2·71	0·953 ±0·024	2·583 ±0·065	0·0478 ±0·0012
C 3	68·11	2·69	0·786 ±0·068	2·114 ±0·184	0·0392 ±0·0034
D	64·01	2·63	1·038 ±0·049	2·730 ±0·132	0·0506 ±0·0025
		Mean P.E. ±0·027			
Cox's Orange Pippin, 1924–25. 3° C. (corrected for interference). 138 days.					
A	68·7	2·74	0·531 ±0·046	1·455 ±0·128	0·0105 ±0·00093
B 1	61·7	2·64	0·747 ±0·069	1·972 ±0·184	0·0143 ±0·0010
B 11	66·7	2·71	0·194 ±0·043	0·526 ±0·117	0·00381 ±0·00085
B 111	72·4	2·78	0·016 ±0·011	0·046 ±0·031	0·00033 ±0·00023
C 1	68·6	2·74	0·618 ±0·061	1·693 ±0·169	0·0122 ±0·0012
C 21	68·0	2·73	0·587 ±0·062	1·603 ±0·171	0·0116 ±0·0013
C 3	62·2	2·65	0·813 ±0·054	2·154 ±0·142	0·0156 ±0·0010
D	73·1	2·79	0·598 ±0·077	1·668 ±0·216	0·0121 ±0·0016
		Mean P.E. ±0·036			
Cox's Orange Pippin, 1925–26. 12° C. 89 days.					
A	62·7	2·46	0·541 ±0·044	1·33 ±0·109	0·015 ±0·0012
B 11	60·6	2·44	0·240 ±0·020	0·59 ±0·049	0·0066 ±0·00055
B 111	67·8	2·53	0·239 ±0·0088	0·60 ±0·024	0·0067 ±0·00027
C 1	64·4	2·44	0·800 ±0·023	1·95 ±0·101	0·022 ±0·0011
C 21	54·5	2·35	0·750 ±0·072	1·76 ±0·187	0·020 ±0·0021
C 3	57·4	2·40	0·520 ±0·075	1·25 ±0·181	0·014 ±0·0020
D	60·9	2·44	0·840 ±0·023	2·05 ±0·062	0·023 ±0·00069
D 2	63·1	2·47	0·353 ±0·052	0·87 ±0·129	0·0098 ±0·0014
		Mean P.E. ±0·033			

The data of rate of advance have been presented again in serial order in Table IV, the most rapidly growing strain being in each case in the first column.

From Table IV it is clear that the two experiments with Cox's Orange Pippin agree exactly in the order of virulence of the strains, when D 2 and B 1 which were not common to the two experiments are left out of account. This agree-

ment is the more striking when it is observed that the growth-rate in the 1925–26 set is very much less than for 1924–25.

Table IV.—Rates of Invasion for the same *Fusarium* Strains arranged in serial order.

	Strains of *Fusarium* and radial advance in centimetres per day.							
Bramley's Seedling, 1925–26. 12° C. 70 days.	D 0·0322 ±0·0020	C 3 0·0296 ±0·0024	A 0·0292 ±0·0021	C 21 0·0248 ±0·0024	C 1 0·0237 ±0·0021	D 2 0·0188 ±0·0010	B 111 0·0179 ±0·0070	B 11 0·0178 ±0·0009
Bramley's Seedling, 1924–25. 12° C. 103 days.	C 1 0·0232 ±0·0017	D 0·0219 ±0·0015	C 21 0·0195 ±0·0012	C 3 0·0192 ±0·0015	B 1 0·0180 ±0·0013	B 11 0·0123 ±0·0013	A 0·0113 ±0·0013	B 111 0·0040 ±0·0011
Cox's Orange Pippin, 1925–26. 12° C. 89 days.	D 0·023 ±0·00069	C 1 0·022 ±0·0011	C 21 0·020 ±0·0021	A 0·015 ±0·0012	C 3 0·014 ±0·0020	D 21 0·0098 ±0·0014	B 111 0·0067 ±0·00027	B 11 0·0066 ±0·00055
Cox's Orange Pippin, 1924–25. 12° C. 54 days.	D 0·0506 ±0·0025	C 1 0·0487 ±0·0030	C 21 0·0478 ±0·0012	B 1 0·0469 ±0·0040	A 0·0424 ±0·0031	C 3 0·0392 ±0·0034	B 11 0·0188 ±0·0018	B 111 0·0050 ±0·0011
Cox's Orange Pippin, 1924–25. 3° C. 138 days.	C 3 0·0156 ±0·0010	B 1 0·0143 ±0·0010	C 1 0·0122 ±0·0012	D 0·0121 ±0·0016	C21 0·0116 ±0·0013	A 0·0105 ±0·00093	B 11 0·0038 ±0·00085	B 111 0·00033 ±0·00023

The agreement between the corresponding Bramley's series in relation to order of virulence is much less marked. For instance C 1 in 1924–25 occupied the first place while in 1925–26 it was fifth in order. Strain A also has changed from being the last but one in 1924–25 to the third place in 1925–26. Similar discrepancies are seen in the set of *Fusarium* strains growing in Cox's Orange Pippin at 3° C. as compared with those at 12° C.

The records made at the time of estimating the rot show that signs of withering were noted in both the sets with Bramley's Seedling and the Cox's at 3° C. In the case of the Bramley's 1924–25 set and the Cox's 3° C., notes with regard to withering were made on each individual apple, and it was possible in these cases to arrive at an estimate by noting the proportion of apples which showed signs of withering. As it seemed possible that the withering had partly accounted for the changed order in these cases, as compared with the Cox's at 12° C., correlations were worked out in each case between the percentage of withered apples in a set and the growth-rates. The results gave for Bramley's 1924–25, $r = -0{\cdot}833$ and for Cox's $r = -0{\cdot}666$. The negative sign in both cases indicates that the growth-rate tends to fall with increasing intensity of withering.

As it was thought that these correlations may have been due to a spurious

effect—since in general the greatest withering occurred with the slowest growing strains, and the possibility of the absorption of water by the living tissue from the dead watery areas could not be neglected—another method of correlation was resorted to. In this case the rates of advance calculated for the set of Bramley's 1925–26 where withering was not so marked, and the set of Cox's 1924–25, 12° C., where withering was not noted, were taken as normal. The ratios of the growth-rates of these sets as compared with corresponding abnormal sets were determined and their ratios correlated with the observed percentage of withering. The correlation coefficients were for Cox's $r = + 0{\cdot}738$ and for the Bramley's $+ 0{\cdot}789$. The positive sign of this result indicates that where the percentage of withering was high, the ratio of the rate of growth in the normal set as compared with the abnormal set tended to rise; or, in other words, in the withered apples the growth-rate of the fungus was suppressed.

As in this method the rates in apples affected with the same strain were compared, there is no question of a differential effect on water loss due to the presence of different amounts of rotten tissue. The evidence seems to point to a real effect of withering on the growth-rate of the fungus, and may possibly arise by competition for water between the fungus and the host tissues, or an increase in concentration of the cell contents. The drying-out of the rotten areas, in cases where "interference" occurs, indicates that absorption of water from the dead tissues can actually occur, and experiments to test the effect of water loss are now in progress.

In order to assess the relative effects of apple variety, of season, and of temperature on the rate of invasion by different strains of *Fusarium*, Fisher's method of analysis of variance (8) was applied to the data. It was assumed that the rate of radial advance is determined by the interaction of two factors, viz., the susceptibility of the apple and the activity of the strains, and also that the rate of advance was proportional to the products of these two factors. For this reason in the process of analysis the logarithms of the values for radial advance were used instead of the actual values recorded. Seven strains were investigated, D 2 and B 1 being omitted since the records for these two strains were not complete. Variety of apple and experimental conditions (season and temperature) yielded five combinations. Thus there were in all 35 combinations of experimental conditions with fungal strains giving 34 degrees of freedom, 7 fungal strains with 6 degrees of freedom, and 5 experimental conditions with 4 degrees of freedom, respectively. The results of the analysis are shown in Table V.

Table V.—Analysis of Variants.

	Degrees of freedom.	Variance.	Mean square.
Strains	6	2·68179	0·44699
E. conditions	4	1·40910	0·35228
Remainder	24	1·61746	0·06740
Total	34	5·70835	—

The 24 remainders give an estimate of the mean discrepancies of individual results from the assumed product formula. The figure corresponds to a standard error of 16·7 per cent. for a single determination. From the figures for probable error of radial advance in Table III it is possible to compare this calculated mean error with the experimental errors actually recorded, and thus to test whether the departures from the product formula were significant. For strain D the mean error recorded in the experiments was 11·4 per cent., for strain C 3 14·0 per cent., while for strain B 111, in which variation in radial advance was greatest, the value of the mean error was 29·7 per cent.

It is clear then that the errors recorded in the experimental data agree very well with the expected discrepancy from values for radial advance based on the assumption that this process is conditioned by the product of activity of fungal strains and susceptibility of apples—susceptibility is taken to be the reciprocal of resistance.

Table V shows further that the differences in strains had the largest effect on rate of advance, but that the experimental conditions were almost as effective. There is no significant difference between these two effects, but both show very high significance (about eight times their mean error) as compared with the random errors.

The effect of experimental conditions can be traced almost entirely to the difference between the experiments at 12° C. and the single experiment at 3° C. The seasons and variety of apple show no significant differences in their effects among themselves. It may be concluded from this that the variety of apple and seasonal effects do not greatly affect the relative virulence of the different strains tested, while there are indications that at different temperatures the amount of infection due to different strains relative to each other may vary. The effect of different levels of temperature on the relative virulence of fungal species has already been observed, and it appears that similar relations may hold for strains of the same species.

This analysis of variance indicates further that in general the effect of the

various fungal strains is due largely to the two strains B 11, B 111, which differ significantly from the rest, while in the data no significant difference among the others is found. Taking the rate of radial advance of the highest strain as 100 and calculating the others as a percentage for each set, the averages for each strain based on all the data are given in the following table (Table VI).

Table VI.—Comparison of Rates of Radial Advance for *Fusarium* Strains.

Strain.	Relative figure of radial advance.	Strain.	Difference in radial advance.
D	94·4±3·3		
C 1	88·7±3·3	D—C 1	5·7±4·7
B 1	87·5±4·6	D—B 1	6·9±5·7
C 21	83·4±3·9	D—C 21	11·0±5·1
C 3	82·6±3·7	D—C 3	11·8±5·0
A	72·1±3·1	D—A	22·3±4·5
D 2	50·5±4·3	D—D 2	43·9±5·4
B 11	39·7±2·6	D—B 11	54·7±4·2
B 111	22·7±1·5	D—B 111	71·7±3·6

The probable errors for each value are also given, together with the probable error of the difference between the rates of advance of the different strains as compared with strain D.

If three times the probable error is taken as significant it is seen that strains D, C 1, B 1, C 21, and C 3 do not differ significantly, while the rest, viz., A, D 2, B 11 and B 111, fall into a separate class. Furthermore, B 11 and B 111 differ significantly from strains A and D 2 and also *inter se*. The strains may then be divided into three groups D to C 3 of high, and A and D 2, medium, and B 1 and B 111 low virulence.

The history of the B strains is very interesting, for with each saltation the virulence is seen to diminish. The same relation holds for D and D 2 and also C 1, C 21 and C 3. In the last case cited the difference in virulence is not high enough to be significant, but the fact that this order is identical in the two experiments with Cox's at 12° C., as well as for the average of all, indicates strongly that, in fact, the virulence of these three strains falls into the order given. In the case of the D strains, the saltant is found in the medium class, while for the B strains in spite of the fact that B 1 is in the highest class, both saltants fall into the lowest class.

A similar relation has been found to hold with certain strains of the section *Discolor* of *Fusarium*, recently investigated in this laboratory by Horne and Mitter (9). It is perhaps significant that, in all cases of saltation whose history

is known, the parent form displays a higher virulence than the strains derived from it, although the fall in virulence may vary greatly in magnitude.

IV.—*Comparison of Variation in Resistance shown by different Populations of Bramley's Seedling Apples.*

Experiments were started in the season 1922–23 to investigate the resistance of apples derived from different localities in relation to a particular fungal species. The variety of apple chosen was Bramley's Seedling and the fungus was *Cytosporina ludibunda*. The apples were obtained from two localities, viz., Burwell, Cambridgeshire (fen soil) and Spalding (silt soil). Batches of 100 apples each were inoculated and stored at Cambridge under constant conditions of humidity and temperature (1° C.). After some months the apples were examined and separated into the classes invaded and not invaded. Since " internal breakdown " (a functional disease encountered when apples are stored under low-temperature conditions) was present, the number showing signs of this trouble was also recorded. The result is given in the following table (Table VII).

Table VII.—Effect of Locality of Origin of Apples on Invasion, 1922–1923.

Locality.	Percentage of apples invaded by *Cytosporina*.	Percentage of apples invaded by *Cytosporina* and affected with internal breakdown.	Percentage of apples with internal breakdown.	Percentage of apples not invaded.	Interval in days.
Burwell (fen)	0	15	77	8	177
Spalding (silt)	32	18	10	40	237

It will be seen that the two categories of Bramley's yielded different results. Thus only 15 of the Burwell apples were invaded, whereas 50 of the Spalding apples showed signs of invasion. These figures are misleading, since of the fen apples which were affected a large percentage gave large amounts of rot, while silt apples showed very small amounts, and indeed all showed " interference." The need for an improvement on the method of assessing the resistance by the mere counting of numbers invaded became obvious at once and led to the development of the technique described in the previous paper (1).

In the following year, 1923–24, apples from six localities were obtained and

stored after inoculation at 3° C. In addition to noting those apples which showed no apparent invasion and those completely rotten, the number in the intermediate class was recorded and measurements were made of the rotten areas. The results as obtained in 1923–24 are recorded in Table VIII.

Table VIII.—Effect of Locality of Origin of Apples on Invasion, 1923–1924.

Locality.	Percentage completely rotten.	Percentage intermediate stages.	Percentage not invaded.	Percentage with internal breakdown.	Interval in days.
Spalding (silt)	3	18	72	98	164
Wisbech (fen).....	3	59	38	—	140
Worcester (old red sandstone)	10	84	6	—	120
Canterbury (gravel)	36	56	8	—	118
Burwell (chalk)	45	55	0	—	122
Burwell (fen)	62	38	0	—	116

Before the new measure (radial advance) could be utilised for this set of data, it was necessary to be able to convert the measurements of area into radial advance. This was done by means of two sets of data collected in the years 1924–25 and 1925–26, relating to Bramley's Seedling from Canterbury and from Burwell, for which both measurements of area and the weights of rotten tissue were recorded; 137 measurements of area and weight records were obtained. The percentage weights of rotten tissue were converted into radial advances from the graph (fig. 1) given in the previous paper (1), and a correlation diagram was set up showing the relation between radial advance (measured as percentage of radius) and the mean diametral arcs of the areas. The regression line was found to be linear, and to be practically identical for the figures derived from the two sets of apples. The correlation coefficient estimated from all the figures was $r = +0{\cdot}924$. From the regression line the percentage radial advance corresponding to any given area could be read off. In this way the data for areas measured in 1923–24 were converted into radial advances, and the results obtained for the apples from the six localities are given in Table IX.

The figures show large differences in the rate of radial advance for apples from different localities, and this confirms the empirical results already presented in Table VIII. Chemical analysis of samples of apples from the same localities gathered at the same time are available and have been published by H. K. Archbold (10) and Janet W. Brown (11). It is therefore possible to investigate the question as to whether chemical composition plays any part in deter-

Table IX.—Data of Invasion in Table VII in terms of Radial Advance.

Locality and soil.	Percentage radial advance.	Average percentage weight rotten.	Days.	Radial advance centimetres per day.
Spalding (silt)	0·0498	< 1·0	164	0·0010
Wisbech (fen)	0·336	3·5	140	0·0084
Worcester (old red sandstone)	0·722	27	120	0·0201
Canterbury (gravel)	0·832	39	118	0·0247
Burwell (chalk)	0·977	60	122	0·0277
Burwell (fen)	1·104	87	116	0·0333

mining the relative rate of advance in the sets of apples. The rate of advance was first correlated with the nitrogen and potassium content, using the fresh weight figures in the papers already referred to. The result is as follows :—

$r_{RN} = + 0{\cdot}7622$ R = radial advance per day.
$r_{RK} = - 0{\cdot}3951$ K = percentage K in fresh weight of apples.
$r_{NK} = - 0{\cdot}2896$ N = percentage N in fresh weight of apples.

There was found to be no correlation with the phosphate content of the apples.

The negative correlation between nitrogen and potassium in the series of apples used indicated the necessity for eliminating the effects of the association of these two factors in determining the correlation of each with radial advance. The partial correlations are shown below :—

$$r_{RN.K} = + 0{\cdot}737 \qquad r_{RK.N} = - 0{\cdot}282.$$

These correlations show that when the effect of the negative correlation of radial advance with potassium content has been allowed for, a large positive correlation of radial advance with nitrogen content is found, while the correlation with potassium is slightly negative, when the effect of nitrogen is eliminated. The high positive correlation of the rate of advance with nitrogen is suggestive, but not significant, since only six pairs of values were available. The negative correlation with potassium is not significant. The results indicate that high nitrogen content of apples enhances the susceptibility to the fungus used, while high potash content tends to increase resistance. The need for further investigation along these lines is evident and further work is now in progress.

V.—*Concerning Resistance to Fungal Invasion in Apples.*

Reviewing the results presented in this and the former communication it is seen that (1) the resistance of the individual apple is of a specific nature ;

(2) apples of a given variety differ in their resistance to different species of fungus and strain of a given fungus; (3) variation in resistance of a given type of apple is associated with the season in which the apples are grown.

From a consideration of these facts, it seems reasonable to suppose that resistance is of a complex nature. It has been shown that resistance may be correlated with chemical composition of apples, and evidence has been presented which indicates that water content also plays a part.

The differences in resistance of varieties can to some extent be correlated with their known differences, such as variation in acidity, the percentage and nature of the sugars, variation in pectic content, and so on. Preliminary experiments to ascertain the effect of acidity have been carried out by growing various species of fungi in extracts of known *p*H value from apples of different varieties. The results, although merely qualitative, do show differences between the behaviour of different fungi when grown in extracts of known acidity. Two experiments were made, one with apple extracts which were made at the time of picking, and a second approximately six months later, with apples from the same original batch which had been kept at 1° C. The result of the first experiment has been published elsewhere (2, p. 50, Table I).

From this experiment it appeared that *Penicillium* and *Botrytis* grew in all the extracts, which agrees well with their known behaviour on apple varieties, while *Cytosporina*, *Fusarium* and *Pleospora* did not grow in any of the Bramley's Seedling extracts, although their behaviour differed among themselves in the extracts from the other varieties. The case of *Cytosporina* is interesting, in that growth was found in some of the Bramley's extracts made after six months' storage, when the *p*H was found to have fallen, which indicates the possibility of changes in resistance due to some tissue change in the apple. The experiments also indicated that there is a limiting level of acidity for particular species beyond which growth cannot proceed.

The relation of the growth of *Pleospora pomorum* in apple extracts of increasing acidity is brought out well by the experimental data obtained in 1921 with extracts of apples kept at 20° C. (3, p. 371). The results show increasing inhibition of growth with increasing acidity.

Further evidence indicating the same effect was obtained by inoculating apple varieties of known *p*H value and noting which varieties were infected with *Pleospora*. Sweet Alford, *p*H 4·1 — 4·6, Dymock Red, *p*H 3·9 — 4·0, Allington Pippin, *p*H 3·05 — 3·35, were invaded, whilst Bramley's Seedling, *p*H 3 — 3·1, was unaffected (see 3, p. 371).

The behaviour of *Pleospora* growing in extracts—(see 7, p. 50, Table I)—

differs from the behaviour of the fungus when inoculated into apples, in that under the latter circumstances no growth is found with Cox's Orange Pippin until the expiry of a considerable period of time (see fig. 8). This may perhaps be related to the ascertained fact that *Pleospora* is practically incapable of inverting cane sugar. The following table (Table X) shows the differences of growth of *Fusarium*, *Pleospora*, and *Cytosporina*, in liquid nutrient media containing various sugars as the source of carbon. Figures for the amount of sugar destroyed and amount of cane sugar inverted are also given.

Table X.—Effect of Sugars on Growth of certain Fungi in Culture Solution.

Fungal species.	Solution.	Weight of mycelium.	Sugar inverted.	Sugar destroyed.
		gms.	mgms.	mgms.
Fusarium	Sucrose + nutrient	0·1187 0·1532	340	45
	Glucose + nutrient	0·1041 0·1177	—	358
	Fructose + nutrient	0·0401 0·0393	—	156
Pleospora pomorum	Sucrose + nutrient	0·0085 0·0108	16·8	9·5
	Glucose + nutrient	0·0552 0·0436	—	125
	Fructose + nutrient	0·0190	—	99
Cytosporina ludibunda	Sucrose + nutrient	0·2148	271·0	33·6
	Glucose + nutrient	0·2083	—	935

The differences between *Fusarium*, *Cytosporina* and *Pleospora* with respect to the inversion of cane sugar are very striking, as is also the different amount of growth made by the three fungi in a cane sugar medium. From such experimental evidence, the importance of the nature of the sugars present and the changes which these sugars undergo cannot be overlooked. It seems possible that the growth of *Pleospora* in the extracts derived from Cox's Orange Pippin in the table previously published ((2), p. 50, Table I) may have been due to inversion of cane sugar during the process of sterilising the medium.

The evidence taken altogether suggests very strongly that the immediate cause of resistance in different varieties of apple may vary; the resistance, for instance, shown by Bramley's Seedling may be due largely to high acidity, while the resistance of Cox's Orange Pippin may be due to the fact that in these apples the ratio of cane to invert sugar is higher than for Bramley's, or to other chemical factors. Indeed the resistance of any one variety, or any

individual apple, will be determined by the interaction of all the known factors, such as, (1) acidity, (2) the nature of the sugars, (3) the percentage of nitrogen, (4) the percentage of potash, (5) the rate of loss of water, as well as possible other factors at present unknown. In so far as the intensity of these factors changes during the period of storage the resistance of individual apples to individual fungi will also show changes.

That such changes do occur is indicated strongly by the data for growth in Cox's Orange Pippin (Table I). The precise formulation of the factor-changes is at present not possible, and awaits more closely correlated data of invasion of apples by specific fungi and concurrent chemical analysis made at various times during storage; an investigation is proceeding along these lines. With our present knowledge it may be stated, as a well ascertained fact, that the acidity falls during the period of storage, so that the time of onset of invasion for a fungus intolerant of acid may be delayed until a critical level of acidity has been passed. Such a case seems to be indicated by the behaviour of *Cytosporina*, where growth did not occur in extracts from Bramley's Seedling apples until the fruit had been stored for a period of six months. Even when invasion occurs early in the period of storage, in Bramley's Seedling, which is characterised by high acidity, the decreasing acid content and consequent lowering of resistance will be reflected in an increasing rate of radial advance.

Experiments now in progress show conclusively that for apples inoculated with *Cytosporina ludibunda* and kept at 20° C. such a difference of resistance exists between those picked early and those picked late in the season. This fall of resistance in the case of Bramley's Seedling contrasts with the indicated rise in resistance for the Cox's Orange Pippin shown in Table II. Early and late picking is found in the case of Cox's to lead to a difference of resistance against the invasion of *Fusarium* and *Pleospora*, but in this case the later picked apples are found to have *a higher resistance*, which is in accordance with the estimate of change in resistance found by the measurements of radial advance. Evidence may be cited for the increase in resistance with the date of picking from early data obtained with *Pleospora pomorum* in 1922–23 (see Table XI).

Table XI.—Effect of Date of Gathering on Invasion by *Pleospora pomorum*.

Date of gathering.	Per cent. invaded.	Days in store.
October 2	69	168
October 26	20	155

The interaction of two such factors as water content and acidity—the high values of the first favouring invasion, and of the second inhibiting invasion; both factors, however, changing with time, may lead to those postulated changes in resistance which were schematically presented in fig. 2, in the attempt to account for the phenomenon of "interference."

It may be claimed for the method adopted in this paper that considerable light has been thrown on the problem of resistance of apples to the invasion of fungi. The difficulty of the problem is apparent, but not insuperable; new lines of work have been opened up, and are being actively pursued, which promise to yield results both of immediate economic importance and of scientific interest.

VI.—*Summary.*

1. The rate of invasion in an apple of a given variety does not remain constant, but may rise or fall in time, according to the variety used and the experimental conditions.

2. A comparison of *Fusarium* strains on various apples shows that the virulence of the strains falls into an order which is independent of the variety of apple and the seasonal changes, but is affected by the temperature of storage. All the saltants examined exhibited a lower virulence than the original strains from which they were derived.

3. The influence of the locality of origin on the resistance of apples of a given variety is shown to be very marked. These differences in resistance can be correlated with differences in chemical composition of the apples.

$$r_{RN.K} = +0{\cdot}737 \qquad r_{RK.N} = -0{\cdot}282$$

R = radial advance.
K = percentage K in fresh weight of apples.
N = percentage N in fresh weight of apples.

4. Resistance to fungal invasion is complex in nature, and depends on many factors. Some of these are water content, acidity, nitrogen content, potassium content. Low water content, high acidity, high potash content and low nitrogen content are found to be associated with high resistance. The converse is also true.

In conclusion the authors wish to express their indebtedness to Prof. V. H. Blackman for the great interest he has taken in this investigation; to various research workers of the Food Investigation Board, both in this Institution and at Cambridge, for assistance in various ways, and to Dr. R. A. Fisher of the

Rothamsted Experimental Station, for his advice on the statistical side of the investigation.

LITERATURE CITED.

(1) Gregory, F. G., and Horne, A. S., "A Quantitative Study, etc." Part I [The foregoing paper].

(2) Horne, A. S., "Factors affecting the Internal Resistance of Apple Tissues to Fungal Attack," 'Rep. Food Investigation Board, 1924. Department of Scientific and Industrial Research, London.'

(3) Horne, A. S., "Fungal Diseases of Stored Apples," 'Rep. Imperial Botanical Conference, London,' p. 363 (1924).

(4) Brown, W., "Studies of the Genus Fusarium. IV.—On the Occurrence of Saltations." 'Annals of Botany,' vol. 40, p. 223 (1926).

(5) Anon., "Parasitic Fungi of Stored Apples," 'Rep. Food Investigation Board, 1922, p. 39. Depart. of Sci. & Indus. Research, London.'

(6) Anon., 'Rep. Food Investigation Board, 1923. Depart. of Sci. & Indus. Research, London.'

(7) Brown, W., "A Study of Forms of Fusarium occurring on Apple Fruit," 'Rep. Imper. Botan. Conf., London,' p. 339 (1924).

(8) Fisher, R. A., 'Statistical Methods for Research Workers, Edinburgh' (1925).

(9) Horne, A. S., and Mitter, J., 'Rep. Food Investigation Board, 1925. Depart. of Sci. & Indus. Research, London.' (*In the Press.*)

(10) Archbold, H. K., "Chemical Studies in the Physiology of Apples. II.—The Nitrogen Content of Stored Apples," 'Annals of Botany,' vol. 39, p. 97 (1925).

(11) Brown, Janet W., "Chemical Studies in the Physiology of Apples. V—Methods of Ash Analysis, and the Effect of Environment on the Mineral Constitution of the Apple," 'Annals of Botany,' vol. 40, p. 129 (1926).

HARRISON AND SONS, Ltd., Printers in Ordinary to His Majesty, St. Martin's Lane.

PROCEEDINGS OF THE ROYAL SOCIETY,
SERIES B.

SUBSCRIPTION PRICE 20 SHILLINGS PER VOLUME.

Printed and published for the ROYAL SOCIETY
by HARRISON & SONS, LTD.,
44—47, St. Martin's Lane, London, W.C.2.